and apoptosis in these cells. Time-lapse videos showed neighboring cells also underwent apoptosis, but expression of Bax and/or Bak was essential to this effect as no bystanders were observed in cells lacking both of these MAC components. In osteosarcoma cell lines, this effect relied upon gap junction intercellular communication, as bystander cell death was abrogated either by pharmacological or molecular inhibition of connexin 43. In contrast, an extracellular pathway seemed to underlie bystander effects in breast cancer cell lines. These results may impact development of novel therapeutic strategies to selectively eliminate tumors or minimize the size of tissue injury in degenerative or traumatic cell death.

222-Pos Board B22
Effect of Ionic Strength on Bax:TBID Mediated Mitochondrial Outer Membrane Permeabilization (MOMP)
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Fluctuations in ionic strength have been documented in apoptosis. While the exact molecular identity of the channels through which proteins permeate across the outer mitochondrial membrane is unclear, it is fairly certain that the channel is large in size and sustained over time. We sought to determine the effect of salt concentration on Bax and tBid in inducing MOMP. While high salt buffer (60mM) facilitated more release of inter-membrane space (IMS) proteins (Adenylate Kinase and Sulfite Oxidase) with Bax and tBid than low salt (5mM HEPES buffer), low salt conditions induced more sustained, real-time permeabilization, as measured by accessibility of exogenous cytochrome c to complex IV. There was a dichotomy between the kinetics of IMS protein release and real-time permeabilization at high salt conditions, while under low salt conditions, they were congruent. The effect of ionic strength on sustained permeabilization is biphasic, with maxima at 10mM. The sustained permeabilization is inducible by diluting the salt concentration to that of low salt, but irreversible by incorporating high salt conditions. This observation is inconsistent with a dynamic channel model sensitive to ionic strength. It seems likely that under low salt conditions, there is a co-operative interaction between Bax molecules in the membrane and those in solution, but the growth of the channel by membrane-bound Bax molecules itself is independent of ionic strength. Supported by a grant from NSF (MCB-0641208).

223-Pos Board B23
Bax, Bcl-xl Exert their Regulation on Different Sites on the Ceramide Channel
Meenu N. Perera, Alicja Bielawska, Zdzislaw M. Szulc, Robert Bittman, Marco Colombini.
Ceramide is a sphingolipid that has been shown to play a vital role in the commitment of a cell to apoptosis. There is increasing evidence that ceramide channels may be the pathway through which cytochrome c is released from mitochondria, a critical step in the apoptotic process. Ceramide content increases in mitochondria upon an apoptotic signal and can form stable channels in mitochondrial membranes which are large enough for the passage of proteins. The Bcl-2 family of proteins regulate apoptosis and have pro-apoptotic members, like Bax, and anti-apoptotic members, like Bcl-xL. These proteins have been shown to directly interact with ceramide channels; Bax directly enlarges ceramide channels and Bcl-xL directly disassembles ceramide channels. The molecular site of interaction of Bax and Bcl-xL with ceramide was probed using structural analogs of ceramide which retain channel forming ability. Mitochondrial outer membrane permeability, and thus channel formation, was assessed using the dynamic cytochrome c accessibility assay. The results indicate that Bax was most sensitive to changes in the head group of ceramide while Bcl-xL was most sensitive to changes in tail length. These changes were not simply an effect of kinetics as longer incubations did not yield different results. Furthermore, when we tested known inhibitors of Bcl-xL, 2-methoxyantimycin A3 and ABT-737, the inhibitory effects of Bcl-xL on ceramide could be reversed. The results obtained in this study paint a very novel picture of how proteins may indeed be able to identify and regulate ceramide channels during apoptosis. Supported by a grant from NSF (MCB-0641208).

224-Pos Board B24
Signaling Complexes Transferred by Outer Mitochondrial Membrane Mixing
David Weaver, Veronica Eisner, Xingguo Liu, Peter Varnai, Laszlo Hunyady, Gyorgy Hajnoczky.
The outer surface of the mitochondrion is home to a host of important signaling complexes. While it has been shown that fusion is an important regulator of mitochondrial health through the mixing of matrix contents and mtDNA complementation, little is known about the mixing of membranes, per se or of membrane associated proteins. Here we demonstrate by a PEG cell-fusion assay that two outer membrane associated signaling proteins–the A kinase anchoring protein, AKAP1 and the pro-apoptotic BCL-2 family member, BAD_are efficiently transferred during membrane mixing events. Using photoactivatible GFP fused to the targeting sequence of AKAP1 in combination with matrix targeted DsRed, we found that such events usually lead to the mixing of matrix contents within seconds, but in a fraction of cases the organelles separate without doing so, indicating a potentially distinct function and mechanism for the mixing of the outer membrane and associated proteins.

225-Pos Board B25
Mitochondrial Fusion Dynamics in Adult Rat Skeletal Muscle
Veronica Eisner, Gyorgy Hajnoczky.
Skeletal muscle metabolism and physiology depends on mitochondria function. Impaired mitochondrial function is associated to myopathies and has been suspected to play a role in alcoholic myopathy. A recently recognized determinant of mitochondrial function is mitochondrial fusion-fission dynamics. Whether mitochondria undergo fusion events in adult skeletal muscle is unknown.
We developed an assay to evaluate mitochondria fusion dynamics in adult rat FDB skeletal muscle fibers and satellite cells-derived myotubes expressing mitochondria targeted DsRed (mtDsRed) and photoactivable GFP (mtPAGFP). We used: (1) in vivo electroporated freshly isolated (2 to 24 h) fibers, (2) in vitro adenoviral infected (a) 4 days old fibers and (b) satellite cells-derived skeletal myotubes. Enzymatically isolated fibers co-expressing mtDsRed and mtPAGFP were imaged by confocal microscopy.
Mito-DsRed fluorescence showed intermyofibillar mitochondria arranged in parallel pair rows following the transverse tubules direction. When we tagged the mitochondria in ~5% of total cellular area with two photon photoactivated-GFP, rapid spreading of GFP fluorescence reveled subsets of interconnected mitochondria. Spreading and fusion events occurred mostly in longitudinal direction. Matrix fusion occurred with a frequency of 0.6 ± 0.1 (n=14, mean $\pm$ SE) and 0.3 ± 0.1 events/min/cell (n=12) in fresh and 4 days old fibers, respectively. Skeletal myotubes displayed 6.4 ± 1.5 events/min/cell (n=4). We further evaluated the mitochondrial fusion dynamics in fresh fibers isolated from ethanol and pair-fed (6 month) rats. Fusion events number decreased 33% in fibers coming from ethanol-fed animals. In vitro incubation of fibers with ethanol (80mM, 48h) induced a 97% decrease in the fusion events number (n=8), regarding to control cells. Thus, adult skeletal muscle intermyofibrillar mitochondria undergo fusion that enables mixing of soluble matrix components. Skeletal muscle cells dynamics is dependent on the differentiation stage. Chronic ethanol exposure significantly suppresses fusion dynamics that might contribute to muscle mitochondria and contractile dysfunction.

226-Pos Board B26
Transient Fusion Maintains Mitochondrial Function in Autosomal Dominant Optic Atrophy Associated with Opa1c.984G>A Mutation
Xingguo Liu, Guy Lenaers, György Hajnóczky.
We have identified two classes of fusion events in mammalian cells: complete fusion and transient fusion. In transient fusion, two mitochondria exchanged soluble intermembrane-space and matrix proteins without equilibration of the integral membrane proteins and reseparated preserving the original morphology. Although Opa1, the inner mitochondrial membrane fusion protein is required for both complete and transient fusions, less Opa1 is sufficient to support transient fusion. To understand the specific role of transient fusion in mitochondrial maintenance, we searched for human conditions that display Opa1 loss. Human mutations in Opa1 are associated with Opa1 depletion and cause autosomal dominant optic atrophy (ADOA). Some Opa1 mutations result in mitochondrial fragmentation but the patients' mitochondrial metabolism is well preserved and the clinical symptoms are mild. We reasoned that transient fusion might be retained and support mitochondrial metabolism in these patients. Here, we studied skin fibroblasts derived from a patient who carries the mutation c.984G>A in the GTPase domain of Opa1 and has only weak visual and metabolic impairments, and fibroblasts of two unaffected individuals. In cells bearing Opa1c.984G>A, the two major Opa1 bands were reduced. The majority of the Opa1c.984G>A fibroblasts showed fragmented mitochondria (partial 45%, complete 17%) compared with elongated mitochondria in the controls. In c.984G>A cells with partially fragmented mitochondria, most fusion events were transient, whereas complete fusion dominated in the control cells. Strikingly, the deltapsi, ATP level and mtDNA content were maintained